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ADHESION MOLECULES

Integrins as signaling molecules and targets for tumor therapy

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Integrins as signaling molecules and targets for tumor therapy. Adhesion molecules include ligands and receptors. Together they provide cells with anchorage and traction for migration, and the receptors also mediate signals that control cell polarity, survival, growth, differentiation and gene expression. Integrins are a major group of versatile adhesion receptors that serve both adhesive and signaling functions. They possess shared and unique specificies both outside and inside the cell. Many of the integrins share an affinity toward the RGD recognition sequence in their extracellular matrix ligands, but are still capable of distinguishing different RGD-containing proteins. The shared signaling pathways are likely to include changes in intracellular Ca^{2+} and PIP2 concentrations, and the activation of protein kinase C and focal adhesion kinase. Examples of integrin-specific signaling include that the $\alpha_v\beta_3$ integrin (vitronectin receptor) can potentiate the effects of insulin and certain other growth factors and that the $\alpha_5\beta_1$ integrin (fibronectin receptor) supports cell survival in serum-free cultures by up-regulating the anti-apoptosis protein Bcl-2. Another integrin function is that some integrins, in particular $\alpha_5\beta_1$, are necessary for fibronectin matrix formation. Overexpression of $\alpha_5\beta_1$, which results in the assembly of additional fibronectin matrix, reduces tumorigenicity of cultured tumor cells. Systemic treatment of tumor-bearing mice with an artificially generated fibronectin matrix suppresses metastasis. These and other findings indicate that the ligand binding and signaling functions of integrins offer targets for new therapeutic approaches.

Cells adhere both to one another and to extracellular matrices. These adhesive interactions are thought to play a major role in the construction of the body plan of multicellular organisms during development, because they can guide cells into their appropriate locations in the body and anchor them there. Adhesion is also important in the maintenance of the body plan; tumor cells are able to loosen their attachment to leave their original location and become lodged at distant sites. Adhesion molecules provide the adhesive recognition specificities and signaling that are necessary for these processes.

Integrins are an important family of adhesion proteins [1–3]. These large, heterodimeric membrane proteins are highly versatile adhesion receptors; they anchor cells, provide traction for cell migration and mediate signals. Integrins display specificity at several levels. First, they are expressed in a cell-type and stage-specific manner. Thus, one group of integrins is associated with migration and proliferation in various types of cells. These "emergency integrins" include $\alpha_5\beta_1$, $\alpha_v\beta_3$ and $\alpha_v\beta_6$. Many other integrins are selectively expressed in a certain cell type or a few cell types. Examples of cell-type specific integrins include $\alpha IIb\beta_3$ in platelets and $\alpha_6\beta_4$ in epithelial cells. Another level of integrin specificity is manifested in their ligand binding. Many of the integrins bind the RGD cell attachment sequence, but they recognize that sequence differentially in the context of various ext racellular matrix proteins, such that some bind primarily to fibronectin and others to vitronectin [3]. At yet another level of specificity, individual integrins mediate distinct signals into the cell's interior.

Integrins have been shown to be signaling molecules capable of generating both common signals and signals that are specific for individual integrins. It has also been found that information can flow in the reverse direction through integrins; their ligand binding activity is regulated from the inside of the cell [4–6].

The increasing understanding of cell adhesion at the molecular level has lead to a number of potential therapeutic applications. The potential of these approaches ranges from implant technologies to anti-thrombotics and cancer treatments [7, 8].

In this article, I will review some new developments in integrin-mediated signaling and matrix assembly. Potential therapeutic applications of these observations will also be discussed.

Outside-in integrin signaling

Integrins communicate with the interior of the cells through connections to the cytoskeleton and by transduction of signals. Integrins are linked to actin filaments, and the link is thought to be through talin, α-actinin and filamin [9–11]. The cytoskeletal binding may be responsible for some of the integrin signals, but integrins also mediate chemical signaling. Moreover, the two systems are interconnected. Some of the integrin-linked signaling molecules, such as paxillin and cortactin [11, 12], are associated with the cytoskeleton and the chemical signaling events elicited by integrin-mediated cell attachment control cytoskeletal assembly [13, 14]. Some of the signaling pathways are common to a number of integrins, whereas others appear to be specific for individual integrins.

Signaling pathways shared by integrins

Integrins that mediate cell-extracellular matrix adhesion connect the matrix to the intracellular cytoskeleton at focal adhesions, which are punctate subcellular structures at the interface of the cell and the substrate or the matrix. In addition to the integrins, various signaling molecules assemble in focal adhesions, resulting in the activation of a number of protein tyrosine kinases, including focal adhesion kinase (FAK) and c-*src*. As a result, focal adhesions are the main phosphotyrosine-containing structures in the cell. The principal phosphoproteins in them include FAK, $p130^{Cas}$, a recently characterized docking protein, and certain cytoskeleton-associated proteins such as paxillin and tensin [15–17], which then transmit signals further to downstream pathways.

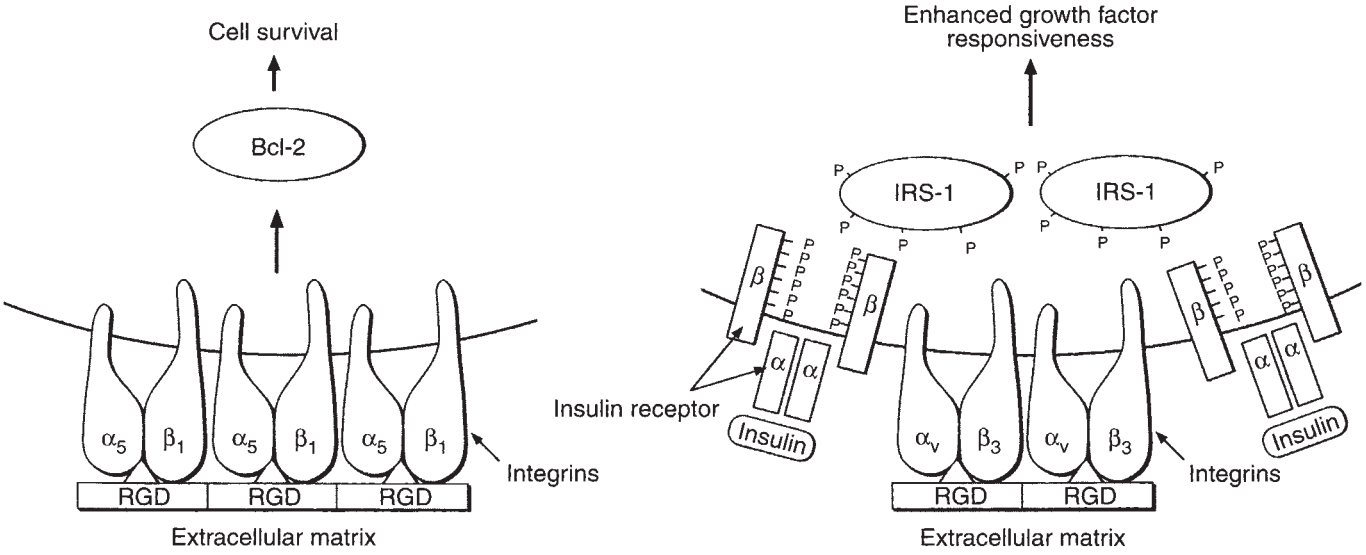

Fig. 1. *Schematic representation of integrin-specific cytoplasmic signaling pathways of the $\alpha_v\beta_3$ and $\alpha_5\beta_1$ integrins.*

In addition, the pathways that appear not to be restricted to any given integrin include increases in intracellular Ca^{2+} and pH, and the activation of the Ras, protein kinase C, phosphoinositol 3′ kinase and phosphoinositol 5′ kinase pathways, and probably a number of others as well [17].

One important function of the integrin signaling pathways is to control anchorage dependence. This term refers to the requirement that normal adherent cells be attached to a substrate to be able to grow. It has been realized recently that anchorage dependence is an integrin-mediated phenomenon. Moreover, it has also been shown that anchorage can mean the difference between life and death to a cell. The early anchorage experiments used fibroblasts, a cell type that becomes growth inhibited in suspension, but remains viable. The new realization is that epithelial and endothelial cells, when detached from substrate, undergo apoptosis [18, 19]. This phenomenon has been named anoikis [19]. To avoid anoikis these cell types will have to attach to a substrate through integrins, attachment through other membrane proteins cannot substitute for the integrins [18–20].

A central role for FAK anchorage dependence is suggested by the recent finding that a constitutively activated FAK variant confers to cells anchorage independence [21]. Moreover, manipulations that increase tyrosine phosphorylation, such as expression of v-*src* or inhibition of phosphatase activity with vanadate, circumvent the anchorage requirement [18, 19]. This is likely to be the explanation for the relative independence from anchorage characteristic of malignantly transformed cells, because oncogenes provide the signals that the cell would otherwise have to derive from substrate attachment.

Signaling by individual integrins

Relatively little attention has been paid to signaling pathways that may be specific for individual integrins. The large cytoplasmic tail of the $\alpha_6\beta_4$ integrin β subunit, which is unique among the integrins, has been shown to bind Shc. This adaptor protein becomes tyrosine phophorylated in an $\alpha_6\beta_4$-dependent manner and is thought to couple the integrin to the Ras pathway through another adaptor, Grb-2 [22]. The $\alpha_v\beta_3$ integrin has also been found to be capable of activating Shc [23], but given the fact that the β_3 and β_4 cytoplasmic tails are completely different, it is likely that the mechanism of the Shc association, and probably its consequences as well, are different for the two integrins. Our laboratory has been working on two signaling systems that appear to be specific for individual integrins, one for $\alpha_v\beta_3$ and the other for $\alpha_5\beta_1$ (Fig. 1).

We have found that the $\alpha_v\beta_3$ integrin cooperates with IRS-1 [24]. IRS-1 is a cytoplasmic signal transduction mediator of the insulin (and insulin-like growth factor, IGF) receptors. IRS-1 is tyrosine-phosphorylated by the activated insulin (and IGF) receptor and as a result binds a number of other signaling molecules [25]. In cells that have adhered to a substrate (such as vitronectin) through the $\alpha_v\beta_3$ integrin, a subset of IRS-1 binds to the integrin. This interaction substantially enhances the growth-stimulating effects of insulin and IGF. No other integrin among the several we have tested interacts with the insulin/IRS-1 system in this manner. A 190 kDa protein that is phosphorylated in tyrosine as a result of PDGF receptor activity also binds to $\alpha_v\beta_3$ [26], suggesting that there may also be cooperation between this integrin and the PDGF pathway. As the $\alpha_v\beta_3$ integrin appears to be selectively associated with endothelial cells undergoing angiogenesis [27], the cooperation of this integrin with insulin could be of significance in the neovascularization of tumors and inflammatory lesions, and in the development of vascular complications of diabetes.

Another recently discovered pathway that also appears to be specific for an individual integrin relates to the ability of the $\alpha_5\beta_1$ integrin to protect cells against apoptosis under certain culture conditions. Testing CHO cells that were engineered by cDNA transfection to express either the $\alpha_5\beta_1$ or $\alpha_v\beta_1$ integrin as their

fibronectin receptor, we found that only the cells that attached through the $\alpha_5\beta_1$ integrin survived in serum-free culture [28]. This response is associated with an elevated expression of the anti-apoptosis protein Bcl-2. Thus, the $\alpha_5\beta_1$ integrin provides a signal for survival in serum-free culture and perhaps under other stressful conditions. Integrins other than $\alpha_5\beta_1$, including $\alpha_v\beta_1$ [28] or $\alpha_v\beta_3$ and various β_1 integrins (unpublished results), fail to rescue cells from apoptosis under these conditions, even though they may support the attachment of the test cells. It is likely that under other conditions cells depend on other integrins for survival [27, 29]. Although we have tested a number of different cell lines for their $\alpha_5\beta_1$ dependency and found them similar, it is also possible that different cell types differ in their integrin dependence under the same conditions. Integrin-mediated stimulation of protease production appears to be coupled to a different integrin in different cell types, $\alpha_5\beta_1$ in some cells and $\alpha_v\beta_1$ in others [30, 31]. A possible physiological significance of the integrin-selective apoptosis phenomenon is that it may prevent cells from attaching to inappropriate places in the body, because attachment through a "wrong" integrin would induce apoptosis.

It will be important to elucidate these integrin-specific signaling pathways at the molecular level. The $\alpha_5\beta_1$ integrin pathways would appear to be particularly important to work out because, as discussed later on in this article, this integrin plays an important role in malignancy.

Inside-out signaling: Regulation of integrin activity

Controlling the activity of integrins that are already expressed at the cell surface provides a fast way for a cell to respond to changing conditions. Thus, platelets effect their own aggregation by activating the $\alpha II_b\beta_3$ integrin, which uses fibrinogen as the glue to attach platelets to one another [5, 32]. Activation of β_2 integrins in leukocytes causes these cells to bind to vessel walls during inflammation [6]. Various types of adherent cells can also modulate the activity of their integrins. In some cases, activation can change the ligand specificity of an integrin. Thus, the $\alpha_2\beta_1$ integrin can be induced from being totally inactive to become a collagen receptor and, further, to acquire the ability to bind laminin [33].

The molecular mechanisms of integrin activation are not well understood, but the process is thought to be mediated by the integrin cytoplasmic domains and involve heterotrimeric G-proteins, phospholipids and protein kinases [34]. Protein kinase C is required for integrins to be active, and activators of this kinase activate integrin-mediated cell adhesion. Recently, a family of cytoplasmic proteins, termed cytohesins, have been discovered and implicated as regulators of β_2 integrin ligand binding activity [35]. R-ras, a member of the Ras/Rho superfamily of small GTPases, has been found to regulate the ligand binding of β_1 and α_v integrins [36]. Understanding the mechanisms of integrin activity regulation will be an important goal for future research, because the ability of adhesion receptors to be regulated in their activity and specificity adds a great deal to the versatility of cell adhesion and because integrin activity modulation could also have therapeutic applications. The prevention of platelet aggregation and leukocyte adhesion are obvious possibilities, but even regulating the amount of fibronectin matrix deposition by cells may be possible, at least under some special circumstances [37]. This latter possibility is an important one, because fibronectin matrix and the main receptor for it, the $\alpha_5\beta_1$ integrin, appear to play a key role in the control of the malignant properties of tumor cells.

Effects of fibronectin matrix and $\alpha5\beta1$ integrin on cells

Normal cells elaborate an extracellular matrix underneath and around themselves. This matrix consists of a variety of proteins, many of which are adhesive and mediate the anchorage of the cells to the matrix. Fibronectin is a prototype adhesive protein that is present in the matrix of many types of cells, where it mediates cell adhesion through a number of fibronectin-binding integrins, including $\alpha_5\beta_1$.

Forced expression of the $\alpha_5\beta_1$ integrin in tumor cells reduces tumorigenicity [38, 39]. Conversely, a decrease in $\alpha_5\beta_1$ expression increases the tumorigenicity of CHO cells [40]. $\alpha_5\beta_1$ is the integrin that controls the assembly of fibronectin matrix, and an increase in $\alpha_5\beta_1$ expression is accompanied by an increase in fibronectin matrix assembly. We have attempted to determine whether the tumor suppressor effect seen upon elevated $\alpha_5\beta_1$ expression could be reproduced with increased fibronectin matrix production.

We found a way of turning soluble fibronectin into fibrils without involving the $\alpha_5\beta_1$ integrin. The fibril conversion was induced by treating fibronectin with a small recombinant fragment from the first type III repeat unit of fibronectin [41]. The mode of action of the fragment, referred to as III1-C, appears to be to interfere with the intramolecular binding interactions that keep fibronectin in its soluble configuration. Once those interactions are disrupted, the molecule undergoes self-assembly into fibrils.

The activated fibronectin that results from the III1-C treatment is tenfold more adhesive to cells than fibronectin insolubilized directly from solution, and hence this material is referred to as superfibronectin (sFN). sFN inhibits cell migration in cell cultures in a similar manner as $\alpha_5\beta_1$ overexpression, suggesting that at least some of the $\alpha_5\beta_1$ effects on tumor cells are secondary to the increased fibronectin matrix formation [41]. The latest results show that sFN has striking anti-metastatic activities *in vivo*. Treatment of tumor cells with sFN *in vitro* renders the cells non-tumorigenic upon subsequent injection into mice. More importantly, systemic treatment of mice with sFN strongly inhibits spontaneous metastasis from subcutaneously implanted tumors [8]. A wide variety of tumor types respond to sFN in this manner, including human breast, colon and ovarian carcinoma, melanoma and osteosarcoma implanted into nude mice. The mode of action of sFN requires further study, but when used to treat cells in suspension, it makes them incapable of adhering to any extracellular matrix substrate [8]. We surmise that sFN may similarly coat tumor cells that are in transit *in vivo*, preventing them from attaching to the sites where metastases would otherwise form, and that the tumor cells incapacitated in this manner would then be susceptible to elimination by natural defense mechanisms. If this postulated mechanism of action is correct, sFN would be acting in the same way as RGD peptides, which inhibit ligand binding by certain integrins [3] and have anti-metastatic activity [42]. However, direct comparison has shown that sFN is more potent in this regard than the peptides [8].

Taken together, the observations discussed above suggest that the fibronectin matrix is primarily responsible for the suppression of malignant properties in cells expressing elevated levels of the

$\alpha_5\beta_1$ integrin. However, the effects of the matrix may in turn be transmitted into the cell by $\alpha_5\beta_1$, although this remains to be demonstrated.

New therapeutic possibilities

One integrin-directed drug, an anti-β3 integrin antibody for the prevention of arterial restenosis, has reached the marketplace so far, but several other integrin-based drugs are under development. For example, peptides containing the integrin-binding RGD sequence [3], and mimics of such peptides that specifically block individual integrins, are being developed as drugs. These compounds target thrombosis ($\alpha II_b\beta_3$ in platelets [7, 32]), osteoporosis ($\alpha_v\beta_3$ in osteoclasts [43]) and tumor angiogenesis ($\alpha_v\beta_3$ in neovascular endothelial cells [44]). The anti-metastatic effect of RGD peptides may offer another target. However, because the metastasis work has been done with peptides that are not selective for any individual integrin, it is not known which of the many RGD-directed integrins mediates the antimetastatic effect. Moreover, as discussed above, sFN may be a more effective compound for this particular application.

In summary, recent work on the integrins has greatly increased our understanding of central cell biological phenomena, such as anchorage dependence, as well as generated a number of possible approaches to new therapies of cancer.

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